

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#11/180

Applicant: Kisiday, et al.

Examiner: Naff, D.

Serial Number: 09/778,200

Art Unit: 1651

Filing Date: February 6, 2001

Attorney Docket: 0492611-0454

(MIT 8813)

ECENED JANO 7 7003 ECHOENTER 1800

Title: PEPTIDE SCAFFOLD ENCAPSULATION OF TISSUE CELLS AND

**USES THEREOF** 

Assistant Commissioner for Patents Washington, DC 20231

Sir:

### RESPONSE TO RESTRICTION REQUIREMENT

In response to the Restriction Requirement mailed July 1, 2002, Applicants elegations 1, claims 1-8.

Please charge any additional fees that may be associated with this matter to our Deposit Account No. 03-1721.

Respectfully submitted,

Monica R. Gerber, M.D., Ph.D. Registration Number 46,724

Choate, Hall & Stewart Exchange Place 53 State Street Boston, MA 02109 (617) 248-5000 Dated: December 31, 2002 3494067\_1.DOC

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner For Patents, Washington, D.C. 2023)

on 12-31-02-

OCT 1 8/30 & X / Seg-

TECH CENTER 1,60

ATTORNEY DOCKET NO. 01997/537001

Certificate of Mailing: Date of Deposit: October 10,3001

I hereby certify under 37 C.F.R. § 1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated above and is addressed to the Assistant

Commissioner for Patents, Washington, D.C. 20231.

TRACEY Simmons

Printed name of person mailing correspondence

Signature of person mailing correspondence

# IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:

John Kisiday et al.

Art Unit:

1614

Serial No.:

09/778,200

Examiner:

Not yet assigned

Filed:

February 6, 2001

Customer No.:

21559

Title:

PEPTIDE SCAFFOLD ENCAPSULATION OF TISSUE CELLS AND

**USES THEREOF** 

Assistant Commissioner for Patents

Washington, D.C. 20231

## PRELIMINARY AMENDMENT

Prior to examination of the above-referenced application, please consider the following amendments and remarks. Please amend the application as follows.

In the Specification:

Please replace Table 1 on page 19, line 1, through page 20, line 9, with the following table that has been re-written in "clean form".

Table 1. Representative Self-Assembling Peptides

Name	Sequence (n>c)	Modulus	Structure	SEQ ID NO
RADA16-I	n-RADARADARADA-c	I	β	1
RGDA16-I	n-RADARGDARADARGDA-c	I	r.c.	2
RADA8-I	n-RADARADA-c	I	r.c.	3
RAD16-II	n-RARADADARARADADA-c	II	β	4
RAD8-II	n-RARADADA-c	II	r.c.	5
EAKA16-I	n-AEAKAEAKAEAKAEAK-c	I	β	6
EAKA8-I	n-AEAKAEAK-c	I	r.c.	7
RAEA16-I	n-RAEARAEARAEARAEA-c	I	β	8
RAEA8-I	n-RAEARAEA-c	I	r.c.	9
KADA16-I	n-KADAKADAKADAKADA-c	I	β	10
KADA8-I	n-KADAKADA-c	I	r.c.	11
EAH16-II	n-AEAEAHAHAEAEAHAH-c	II	β	12
EAH8-II	n-AEAEAHAH-c	II	r.c.	13
EFK16-II	n-FEFEFKFKFEFEFKFK-c	II	β	14
EFK8-II	n-FEFKFEFK-c	I	β	15
ELK16-II	n-LELELKLKLELELKLK-c	II	β	16
ELK8-II	n-LELELKLK-c	II	r.c.	17
EAK16-II	n-AEAEAKAKAEAEAKAK-c	II	β	18
EAK12	n-AEAEAEAEAKAK-c	IV/II	$\alpha/\beta$	19
EAK8-II	n-AEAEAKAK-c	II	r.c.	20
KAE16-IV	n-KAKAKAKAEAEAEAEA-c	IV	β	21
EAK16-IV	n-AEAEAEAEAKAKAKAK-c	IV	β	22
RAD16-IV	n-RARARARADADADADA-c	IV	β	23
DAR16-IV	n-ADADADADARARARAR-c	IV	$\alpha/\beta$	24
DAR16-IV*	n-DADADADARARARA-c	IV	$\alpha/\beta$	25
DAR32-IV	n-(ADADADADARARARAR)-c	IV	$\alpha/\beta$	26
EHK16	n-НЕНЕНКНКНЕНЕНКНК-с	N/A	r.c.	27
EHK8-I	n-НЕНЕНКНК-с	N/A	r.c.	28
VE20*	n-VEVEVEVEVEVEVEVEVEVEVE		β	29
RF20*	n-RFRFRFRFRFRFRFRFRFRF-c		β	30

<sup>&</sup>quot; $\beta$ " denotes beta-sheet; " $\alpha$ " denote alpha-helix; "r.c." denotes random coil; "N/A" denotes not applicable. \*Both VE20 and RF20 form a beta-sheet when they are incubated in a solution containing NaCl; however, they do not self-assemble to form macroscopic scaffolds.

Please replace Table 2 on page 21, lines 17 to 25, with the following table that has been re-written in "clean form".

AZ

Table 2. Representative Peptides for Cross-Linking Study

Name	Sequence (N>C)	SEQ ID NO
RGDY16	RGDYRYDYRYDYRGDY	31
RGDF16	RGDFRFDFRFDFRGDF	32
RGDW16	RGDWRWDWRWDWRGDW	33
RADY16	RADYRYEYRYEYRADY	34
RADF16	RADFRFDFRFDFRADF	35
RADW16	RADWRWDWRWDWRADW	36

Please replace Table 3 on page 22, lines 14 to 21, with the following table that has been re-written in "clean form".

A3

Table 3. Representative Peptides for Enzymatic Cleavage Study

Name	Sequence (N>C)	<u>SEQ ID NO</u>
REEE	RGDYRYDYTFREEE-GLGSRYDYRGDY	37
KEEE	RGDYRYDY <u>TFKEEE-GLGS</u> RYDYRGDY	38
SELE	RGDYRYDYTASELE-GRGTRYDYRGDY	39
TAQE	RGDYRYDYAPTAQE-AGEGPRYDYRGDY	40
ISQE	RGDYRYDY <u>PTISQE-LGQRP</u> RYDYRGDY	41
VSQE	RGDYRYDYPTVSQE-LGQRPRYDYRGDY	42
, 545		

Please replace the paragraph on page 23, line 22, through page 24, line 14,

with the following paragraph that has been re-written in "clean form".

At

A peptide with the amino acid sequence n-KLDLKLDLKLDL-c (SEQ ID NO: 43) (KLD12) was synthesized using a peptide synthesizer (Applied Biosystems) and lyophilized to a powder. A 0.5% peptide casting solution was obtained by dissolving KLD12 in a solution of 295 mM sucrose and 1 mM HEPES. Freshly isolated chondrocytes from bovine calf femoropatellar groove cartilage were re-suspended in the casting solution at a concentration of  $15 \times 10^6$  cells/ml. The suspension was injected into a

GAH AY casting frame consisting of a 40 x 40 x 1.5 mm window supported on both faces by filter paper and a porous mesh. The casting frame was placed in a 1 X phosphate-buffered saline (PBS, which contains 150 mM NaCl and 10 mM sodium phosphate at pH 7.4) bath for 15 minutes to induce the self-assembly of the peptides into a scaffold. Preferably, the cells are incubated in the sucrose solution for less than 5 minutes, or more preferably for less than 1 minute, before PBS is added. If desired, formation of a peptide scaffold may be confirmed using phase-contrast microscopy. As a control, cells were also suspended into warm agarose (2% solution, w/w), injected into the casting frame, and placed into a cold 1 X PBS bath for 5 minutes. Both the peptide and control agarose gels were maintained in DMEM media (Gifco) plus 10 % FBS, which was changed every other day.

Kindly insert the enclosed sequence listing at the end of the application.

#### **REMARKS**

The specification has been amended to provide a unique sequence identification number for each amino acid sequence within the specification. The attached sequence listing has also been inserted into the application. No new matter is introduced by any of the amendments.

Date: October 10, 200/

1. 1 Mbr. 1 July

Respectfully submitted,

Reg. No. 39,109

Clark & Elbing LLP 176 Federal Street Boston, MA 02110

Telephone: 617-428-0200 Facsimile: 617-428-7045

\\Clark-w2k1\documents\01997\01997 537001 Preliminary Amendment.wpd

21559 PATENT TRADEMARK OFFICE

. 100114 11001 11001 E1101 G1116 1911 1991